NEW YORK ENTOMOLOGICAL SOCIETY
LXXXVI(1), 1978, pp. 56–62

THE GLYCOPROTEINS OF INSECT CELLS IN CULTURE

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Abstract.—Goldstein, Neil I., and McIntosh, Arthur H., Waksman Institute of Microbiology, Rutgers University, The State University of New Jersey, P.O. Box 759, Piscataway, N.J. 08854. Present address of senior author: The Wistar Institute, 36th Street at Spruce, Philadelphia, Pa. 19104.—Insect cells in culture synthesize glycopeptides and shed these molecules into the medium. The profiles of ^{14}C-glucosamine labelled proteins, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), show both quantitative as well as qualitative differences between the insect lines studied.

This research was supported in part by National Science Foundation Grant BMS 74-13608.

Received for publication 13 June 1977.

Introduction

Glycoproteins are important macromolecular constituents of cells, occurring in both intra- and extracellular membranes (Yamada and Weston, 1974; Chiarugi and Urbano, 1973). The glycoproteins of the cell's plasma membrane play important biological roles, being involved in active transport, cell to cell recognition, and as viral and hormonal receptor sites (Singer, 1974; Marchesi et al., 1976). The membrane has been shown by histochemical techniques to contain or be closely associated with glycoprotein material (Gasic and Gasic, 1963). Of further interest are the qualitative and quantitative changes which have been reported for glyproteins of cells transformed by viruses and chemical carcinogens (Perdue et al., 1972; Warren et al., 1973). Recent work by Hynes (1976) has shown that a large molecular weight membrane glycoprotein (called LETS protein or large external transforming sensitive protein) is greatly reduced in transformed fibroblasts when compared to the normal parents and may be involved in the adhesion of these cells to the substratum (Hynes, 1976).

Although glycoproteins occur in such diverse cell types as sponge, yeast, plant, and vertebrate cells, most of the glycoprotein studies have involved the latter cell type. In view of the rapid developments and interest in the field of insect cell culture (Maramorosch, 1976), and the limited utilization of insect cell lines, these studies were undertaken to determine whether glycoproteins are normal components of insect cells. In addition, the cellular glycoproteins were separated by polyacrylamide gel electrophoresis (PAGE) in order to differentiate the different species of glycoproteins.

Table 1. Acid precipitable material from insect cells.[a]

	CPM/MG protein		
Cell line	In medium	Trypsinate	Trypsin-extracted cells
AE	31,990 ± 4,499[b]	81,828 ± 19,022	185,771 ± 32,613
SF	32,277 ± 5,145	37,595 ± 8,688	81,281 ± 22,755
CP	23,230 ± 5,525	28,241 ± 8,973	68,107 ± 11,682

[a] Aliquots of the medium, trypsinate, and trypsin-extracted cells isolated from cells grown in radioactive medium were treated for 15 minutes with cold TCA (final concentration = 20% TCA). The precipitated material was collected on filter pads using vacuum filtration. The pads were dried overnight at room temperature and counted by liquid scintillation spectrometry.

[b] Mean ± S.D. based on at least 2 experiments using replicate samples.

Materials and Methods

Cell lines and medium.—The lepidopteran insect cell lines *Spodoptera frugiperda* (SF, IPLB-21), *Laspeyresia pomonella* (CP-169) and the dipteran cell line *Aedes aegypti* (AE) were grown at 28°C in monolayer culture using TC199-MK medium (for SF and CP) (McIntosh et al., 1972) and M & M medium (for AE) (Mitsuhashi and Maramorosch, 1964).

Metabolic labelling of glycoproteins.—Cells from confluent 75 cm^2 T-flasks were removed with trypsin (0.25%), pelleted at 1,000 × g, and 5×10^5 cells resuspended in growth medium containing either 2.8 μ Ci/ml of 3H glucosamine (sp. act. 7.5 ci/m mole) (for plating experiments) or 1.5 μ Ci of ^{14}C glucosamine (sp. act. > 200 m Ci/m mole) (for PAGE). The cells, in 4 ml of medium, were dispensed into several 25 cm^2 T-flasks and incubated at 28°C for 72 hours. At the end of this time period, all of the cells were observed to be in late log phase (non-confluent).

TCA plating procedures.—The medium was asperated, removed from those flasks which were to be used in these experiments and the monolayer washed three times with Hanks' balanced salt solution (HBSS). Four ml of fresh, unlabelled medium were added to each flask and the cells incubated for an additional 24 hours at 28°C. The medium was then removed from each T-flask and the floating cells pelleted by centrifugation. The cells remaining in monolayer were treated with 2 ml of 0.25% trypsin for 30 minutes to remove the exposed surface proteins (trypsinate). The trypsin-extracted cells were pelleted and washed 3× with HBSS. To dissolve the pelleted cells, 0.1 NaOH was added. An aliquot was then removed for protein determination by the method of Lowry et al. (1951).

To a 0.5 ml aliquot of medium were added 2 ml of cold 20% trichloroacetic acid (TCA). To the trypsinate and the trypsin-extracted cells, enough cold 100% TCA was added to bring the concentration to 20%. The sam-

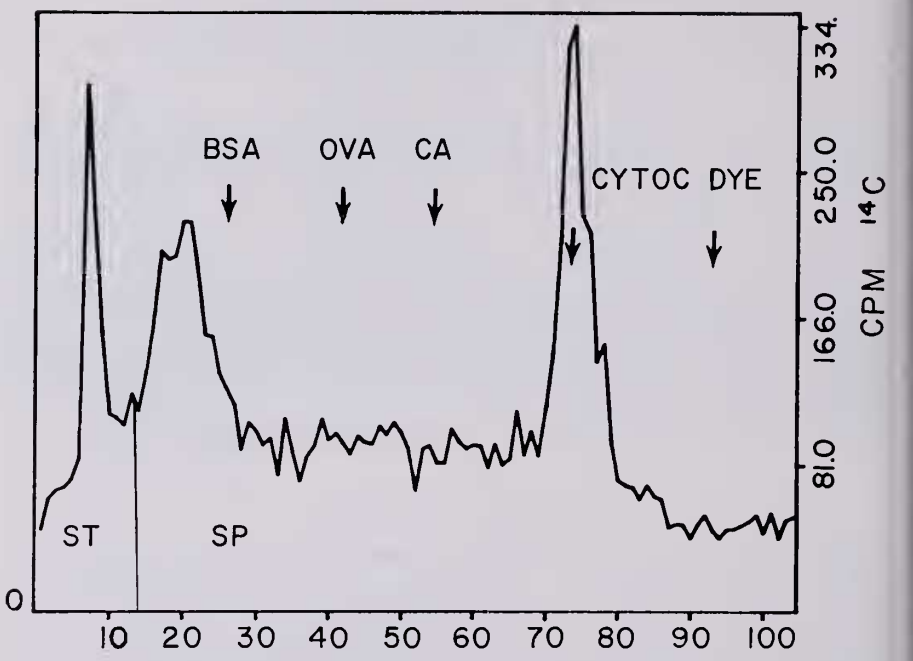

Fig. 1. Electrophoresis of ^{14}C-glucosamine labelled glycoproteins from AE cells. The glycoproteins were labelled, isolated, and electrophoresed as described in the text. Bovine Serum Albumen (BSA), Ovalbumen (OVA), Carbonic Anhydrase (CA), and Cytochrome C (CYTO C) were run as standards. The tracking dye (DYE) was noted.

ples were vortexed and kept at 4°C in an ice bath for 15 minutes. The TCA precipitable material was collected on filter pads using vacuum filtration, washed 5× with cold 5% TCA, dried overnight at room temperature, and counted by liquid scintillation procedures using Aquasol as the cocktail. The amount of glucosamine-labelled material was expressed as counts per minute per mg of protein.

Polyacrylamide gel electrophoresis.—The cells to be isolated for PAGE were washed in monolayer 3× with HBSS, scraped with a rubber policeman, and pelleted at 1,000 × *g*. To the pellet was added 300 λ of PAGE sample buffer containing 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol. Before electrophoresis, the samples were heated for 3 minutes in a boiling water bath. About 25,000 cpm per sample were layered on the gel. The following proteins were run as standards: bovine serum albumen (MW = 66,000); ovalbumen (MW = 46,000); carbonic anhydrase (MW = 31,000); cytochrome C (MW = 12,000). Electrophoresis was carried out in a slab gel system consisting of a 5% stacking gel (pH 6.8) and a 10% separating gel (pH 8.8) (Laemmli, 1970). The samples were allowed to migrate 9.5

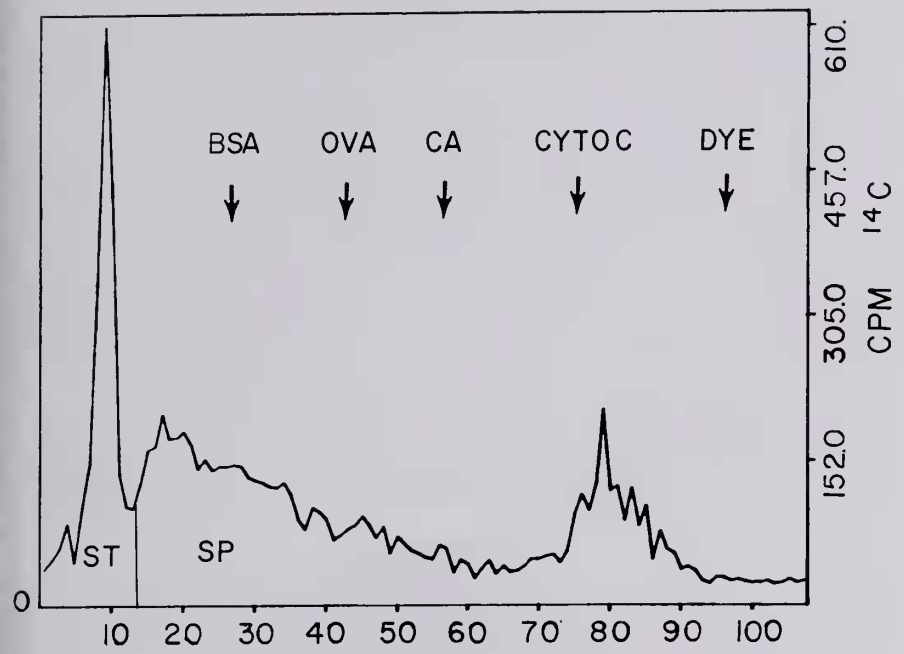

Fig. 2. Electrophoresis of ^{14}C-glucosamine labelled glycoproteins from SF cells. See Fig. 1 for legend description.

cm. The gel was stained with 0.05% Coomassie brilliant blue dye in H_2O, glacial acetic acid and isopropyl alcohol (13:2:5, v/v), and destained in 10% acetic acid. The gel was photographed and each sample slot cut and sectioned into 1 mm slices using a Joyce Loebel Gel slicer. Slices were placed in glass mini vials, solubilized with a homemade cocktail (5% Protosol; 1% H_2O; 4 g PPO; 50 mg POPOP per liter of toluene) and counted by liquid scintillation spectrometry.

Results and Discussion

The results of our experiments show that the three insect cell lines can synthesize glucosamine-labelled proteins which are found in the plasma membrane and are shed into the medium (Table 1). Among the three lines, AE synthesized the greatest quantity of cellular and membrane APM when compared to SF and CP (Table 1). This is probably related to the different growth properties of the cell lines. AE grows to the highest maximum cell density (36×10^6) compared to either SF (17×10^6) or CP (16×10^6) in 25 cm^2 T-flasks (McIntosh, 1976). In addition, it is interesting to note that the amount of APM released into the medium was constant among the three cell lines. This "shed" material probably results from the rapid

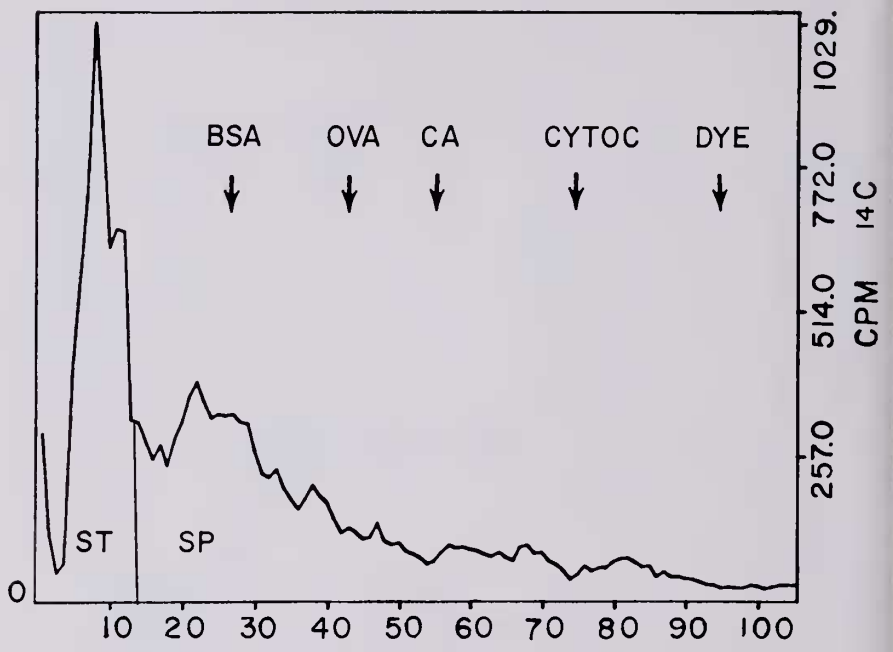

Fig. 3. Electrophoresis of ^{14}C-glucosamine labelled glycoproteins from CP cells. See Fig. 1 for legend description.

turnover of cell surface membrane proteins (Gahmberg and Hakomori, 1974). It would appear that the rate of membrane turnover is the same for the three insect cell lines.

The possibility that these insect glycopeptides might represent non-specifically absorbed, labelled serum proteins (Angello and Hauschka, 1974) was tested. Medium to which 2 μ Ci of ^{3}H-glucosamine had been added and incubated for 72 hours at room temperature was extensively dialyzed in distilled water and $>1 \times 10^{5}$ cpm added to replicate flasks of each cell line for 72 hours. The medium was changed, cold medium added and the cells processed as described in the Materials and Methods section. It was observed that the amount of radioactivity in samples treated in this manner was no higher than background values. Therefore, it seems unlikely that serum proteins are responsible for any counts observed.

Whole cell proteins and glycoproteins were characterized by SDS-PAGE. Proteins separated by electrophoresis can be visualized by staining with Coomassie blue dye. The profiles of the ^{14}C-glucosamine-labelled proteins are shown in Figs. 1–3. In all 3 cell lines, there is a major peak in the stacking gel and a region of peaks in the BSA molecular weight area of the separating gel; in AE and SF, a major peak near the cytochrome C

marker was also observed. Several major conclusions from the data can be summarized: 1) A major peak in the stacking gel region quantitatively different between the 3 cell lines; 2) an area of peaks in the molecular weight range 125,000–50,000; 3) a qualitative difference between CP, on the one hand and AE and SF, on the other in the 12,000–20,000 molecular weight area. It must be noted that molecular weight determinations of glycoproteins by SDS-PAGE are not accurate (Segrest and Jackson, 1972).

The function of insect glycopeptides is unknown. It seems probable that they play a role similar to that in mammalian cells; for example, cell-cell interaction (Spiro, 1970). In addition, there is some evidence that glycoproteins are responsible for the ability of tissue culture cells to attach to a substratum. In general, most insect cells do not attach firmly to culture vessel surfaces and are easily removed from T-flasks by simple shaking. We are presently investigating this phenomenon in relation to insect glycopeptides. In addition electrophoresis of glycopeptides may serve as a useful technique in aiding in the identification of insect cell lines.

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